DRUG DELIVERY — LIQUID CRYSTALS IN

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DEFINITION AND FORMATION OF LIQUID CRYSTALS

Definition

The liquid crystalline state combines properties of both liquid and solid states. The liquid state is associated with the ability to flow, whereas the solid state is characterized by an ordered, crystalline structure (1). Crystalline solids exhibit short as well as long-range order with regard to both position and orientation of the molecules (Fig. 1a). Liquids are amorphous in general but may show short-range order with regard to position and/or orientation (Fig. 1b). Liquid crystals show at least orientational long-range order and may show short-range order, whereas positional long-range order disappears (Fig. 1c) (2). Accordingly, liquid crystalline phases represent intermediate states and are also called mesophases.

A pre-requirement for the formation of liquid crystalline phases is an anisometric molecular shape that is generally associated with a marked anisotropy of the polarizability. Molecules that can form mesophases are called mesogens. Depending on the molecular shape, rod-like mesogens form calamitic mesophases and disc-like mesogens form discotic mesophases. Rod-shaped molecules are often excipients of drugs (e.g., surfactants). Even drug compounds themselves (e.g., the salts of organic acids or bases with anisometric molecular shape) fulfill the requirements for the formation of calamitic mesophases.

Formation

Starting with the crystalline state, the mesophase is reached either by increasing the temperature or by adding a solvent; accordingly, a differentiation can be made between thermotropic and lyotropic liquid crystals, respectively. As with thermotropic liquid crystals, a variation of temperature can also cause a phase transformation between different mesophases with lyotropic liquid crystals.

Thermotropic liquid crystals

Calamitic mesophases were the first liquid crystals to be found more than 100 years ago. In 1888, the botanist Friedrich Reinitzer observed birefringence of cholesteryl esters after melting (3) and contacted the physicist Otto Lehmann, a specialist in crystallization microscopy, who interpreted the birefringence of the molten cholesteryl esters as a parallel orientation of molecules within a liquid crystal, a new kind of state (4). However, these cholesteric liquid crystals exhibit not only a parallel orientation of the anisometric molecules, but the director of the orientation rotates layer by layer in a right- or left-handed helix (Fig. 2b). The layer distance where a 360° rotation has been performed is called pitch, which is often in the magnitude of the visible light. This phenomenon, as well as the variation of pitch with temperature, is responsible for the characteristic color play of cholesteric liquid crystals. Cholesterics require chirality either of the mesogen itself or on addition of a mesogen.

The nematics are similar to cholesteric liquid crystals in having just orientational long-range order, with the deviation that the director of the preferred orientation does not rotate (Fig. 2a). If, however, a chiral mesogen is dissolved in a nematic liquid crystal, the latter will transform into a cholesteric liquid crystal.

Calamitic mesophases with parallel orientation of the molecules, which are additionally arranged in layers, are called smectic liquid crystals (Fig. 2c-e). The layer plane may be oriented either perpendicular or tilted to the long axes of the molecules. Furthermore, the molecules may be arranged regularly within the layer (e.g., in a hexagonal arrangement), thus forming a three-dimensional lattice. As opposed to crystals, the smectic liquid crystalline state enables rotation of the molecules around their long axes. Different smectics may be distinguished in the basis of a variety of arrangements.

Phase transitions occur with increasing temperature, for example, crystalline to smectic C to smectic A to nematic to isotropic, or crystalline to nematic to isotropic. These examples demonstrate that not all possible transitions necessarily occur. Depending on the number of mesophases occuring, thermotropic mono-, di-, tri-, or tetra-morphism may be distinguished.

Discotic liquid crystals arise from disc-shaped molecules as nematic or cholesteric mesophases. Their

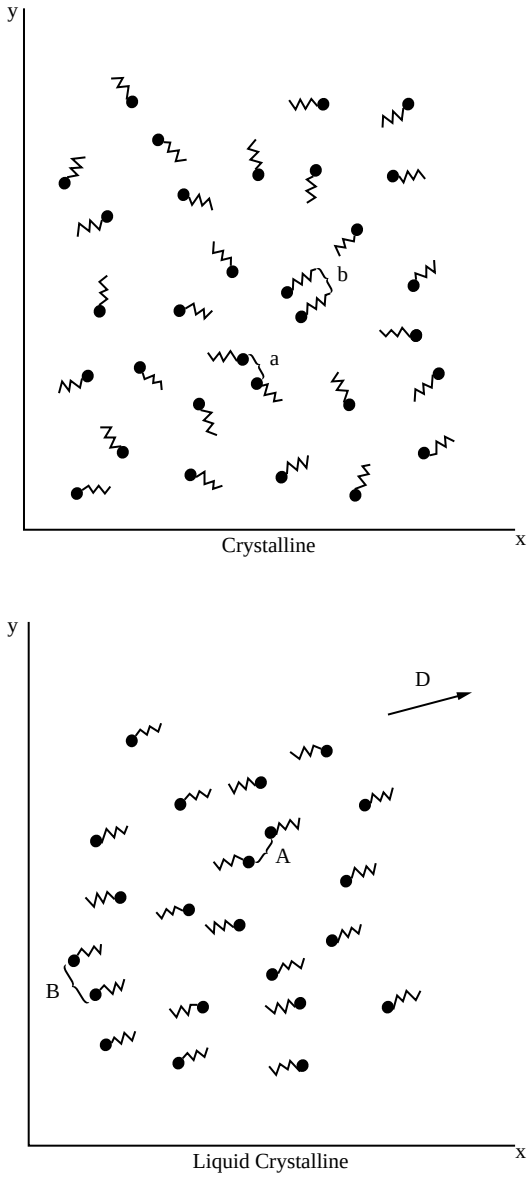


Fig. 1 Two-dimensional representation of short-range order (a, b) and long-range order (A, B) in the crystalline and liquid crystalline states. (From Ref. 2.)

structural characteristics are similar to that of their respective calamitic mesophases, that is, the normals of the discs are oriented parallel. Instead of the smectic mesophases, discotic columnar liquid crystals arise from stapling the discs one on the other. The columns of the discotic columnar mesophase form a two-dimensional lattice that is in either a hexagonal or a rectangular modification. In addition, the columns may or may not be tilted (Fig. 2f and 2g).

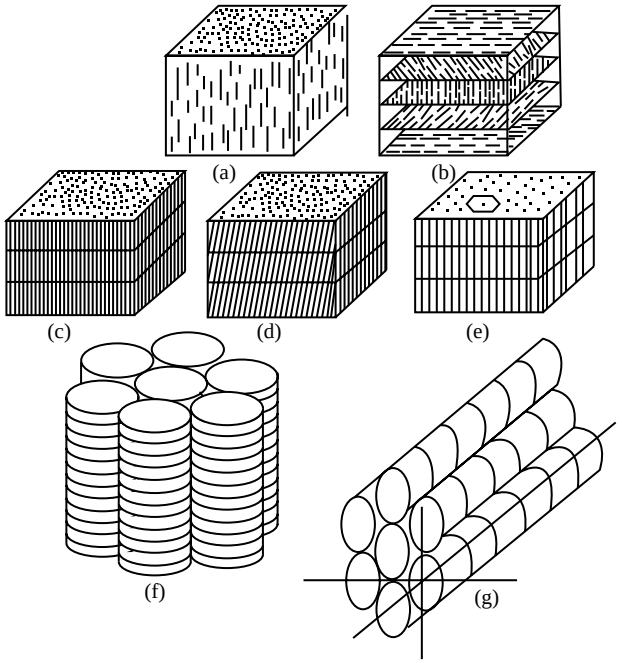


Fig. 2 Schematic representation of different calamitic and discotic thermotropic liquid crystals. (a) nematic; (b) cholesteric; (c–e) smectic; (f) columnar hexagonal; (g) columnar hexagonal tilted. (a–e: Adapted from Ref. 5; f and g: Adapted from Ref. 6.)

Lyotropic liquid crystals

Lyotropic liquid crystals differ from thermotropic liquid crystals. They are formed by mesogens that are not the molecules themselves but their hydrates or solvates as well as by associates of hydrated or solvated molecules. In presence of water or a mixture of water and an organic solvent as the most important solvents for drug molecules, the degree of hydration or solvation depends on the amphiphilic properties of a drug molecule. Hydration—and solvation—of the mostly rod-shaped molecule results in different geometries such as cone and cylinder (Fig. 3) (7).

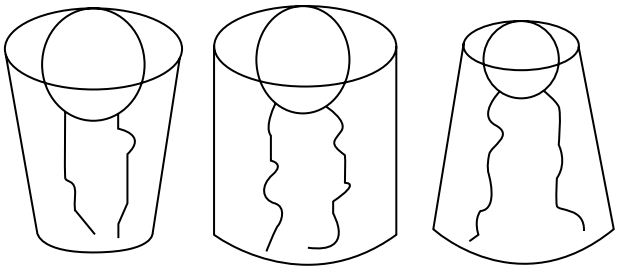


Fig. 3 Geometry of hydrated molecules—cylinders associate to a lamellar liquid crystal, cones to a hexagonal and an inverse hexagonal one. (Adapted from Ref. 7.)

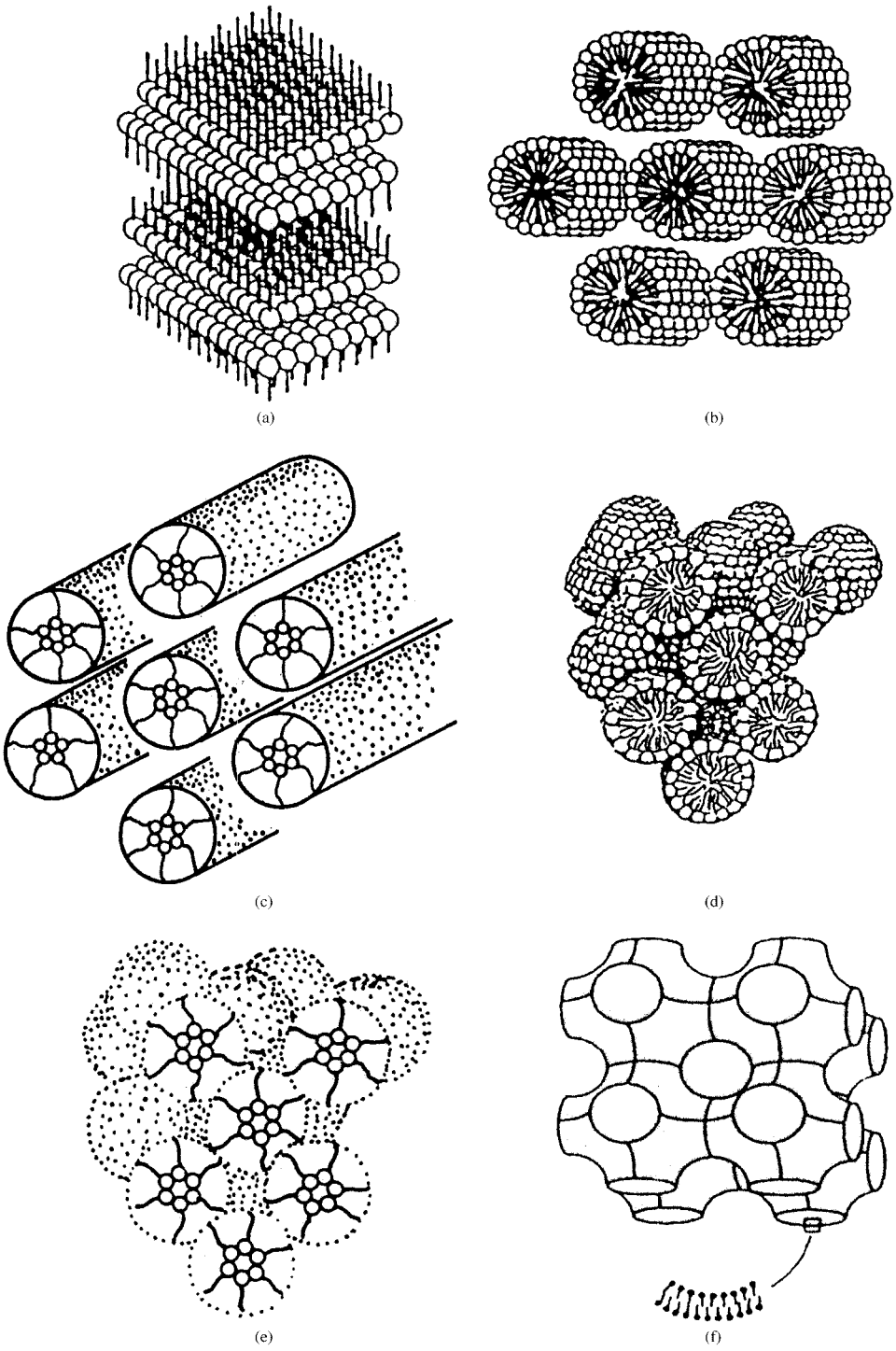


Fig. 4 Molecular structure of lyotropic liquid crystals. (a) lamellar; (b) hexagonal; (c) inverse hexagonal; (d) cubic type I; (e) inverse cubic type IV; (f) cubic type II. (a, b, and d: Adapted from Ref. 8; c: Adapted from Ref. 9; e: Adapted from Ref. 10; f: Adapted from Ref. 11.)

Cylinders arrange in layers; this results in a lamellar phase with alternating polar and nonpolar layers (Fig. 4a). Water and aqueous solutions can be included in the polar layers, resulting in an increase of layer thickness. Analogously, affinic molecules can be included in the nonpolar layers. In addition to the increased layer thickness of the lamellar phase, lateral inclusion between molecules is also possible with an increase in the solvent concentration, which transforms the rod shape of the solvated molecules to a cone shape (Fig. 3), thereby leading to a phase change. Depending on the polar or nonpolar character of the solvating agent and the molecule itself, the transition results in a hexagonal or an inverse hexagonal phase (Figs. 4b and 4c).

The hexagonal phase is named after the hexagonally packed rod micelles of solvated molecules, whereby their polar functional groups point either to the outside (Fig. 4c) or to the inside of the structure (Fig. 4, inverse hexagonal phase). In the hexagonal phase, the additional amount of water or unpolar solvent that can be included is limited. As the molecular geometry changes further during solvation, another phase transformation to a cubic form (type I) or inverse cubic form (type IV) takes place, consisting of spherical or ellipsoidal micelles and/or inverse micelles (Figs. 4d and 4e).

In addition to the cubic and/or inverse cubic forms described previously, further transitional forms exist between the lamellar phase and the hexagonal mesophase (cubic, type II) or inverse hexagonal mesophase (cubic, type III) (12). In contrast to the discontinuous phases of types I and IV, cubic mesophases of type II and type III belong to the bicontinuous phases (Fig. 4f). A range of lyotropic mesophases are possible, depending on the mesogen concentration, the lipophilic or hydrophilic characteristics of the solvent and the molecule itself (Table 1). However, not all theoretically possible mesophases may occur in practice.

Liposomes

With some molecules, a high concentration results in a lamellar phase, but no additional mesophases are formed if the concentration is reduced. The lamellar phase is dispersed in the form of concentric-layered particles in an excess of solvent (water or aqueous solution). This results in a vesicular dispersion. If the mesogenic material consists of phospholipids, the vesicular dispersion is called a liposomal dispersion (13). In principle, liposomes may be dispersed in oily continuous media too. However, the latter systems are of minor interest in drug formulation.

Liposomes consist of many or only few phospholipid bilayers, or just one bilayer (Fig. 5). Based on this, multilamellar vesicles (MLV), oligolamellar vesicles (OLV), small unilamellar (SUV), and large unilamellar vesicles (LUV) can be distinguished. Furthermore, multivesicular liposomes (MVL) may be formed.

The polar character of the liposomal core enables the encapsulation of polar drug molecules. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer depending on their affinity for the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in Niosomes^{TM}. The term sphingosomes is suggested for vesicles from sphingolipids. However, nomenclature is not consistent; the term liposomes is used as a general term, although vesicles would be a better term.

A standard manufacturing procedure of liposomes is the film-forming method. Prior to film formation, the phospholipids are dissolved in an organic solvent. By rotational evaporation of the solvent, a thin multilayered film of phospholipids develops at the inner wall of the vessel. Redispersion of this film in water or aqueous buffer results in the formation of vesicles. The size of the vesicles and the number of bilayers vary. Hence, further manufacturing steps are needed to obtain defined vesicular dispersions with a sufficient shelf-life.

To reduce vesicle size and the number of bilayers, high-pressure filtration via polycarbonate membranes or high-pressure homogenization in a French press or in a microfluidizer are appropiate manufacturing procedures. Sonication may also be applied, although the obtained dispersion does not have the same particle sizes.

Alternatively, injection method and reverse-phase dialysis are appropiate procedures for the formation of SUV and LUV. Freeze–thaw procedures enable drug loading of the liposomes and also offer an evaluation of the stability of the vesicular dispersion. For interested readers, general reading is recommended (13, 14).

Liquid Crystal Polymers (LCP)

Both thermotropic and lyotropic liquid crystal polymers exhibit characteristic features with regard to their microstructure (15, 16). Anisometrical monomers such as rods or discs are connected to chains in an appropiate manner. These anisometrical monomers are considered to be the mesogens and may be part of main chain LCP, side chain LCP, or of both types together (Fig. 6). Between the mesogens are located flexible spacers of nonmesogenic character. Sufficient flexibility is a prerequisite for liquid crystal formation, with an increase in either temperature or solvent concentration.

Table 1 Possible transitions of lyotropic liquid crystals

Micellar	← →	Hexagonal	← →	Lamellar	← →	Inverse hexagonal	← →	Inverse micellar
	Cubic I		Cubic II		Cubic III		Cubic IV	
Lipophilicity of the solvent and/or the amphiphilic compound (AC)	→				AC concentration		←	

(Modified from Ref. 12.)

METHODS FOR CHARACTERIZATION OF LIQUID CRYSTALS

Methods appropriate for the investigation and characterization of lyotropic liquid crystals are frequently used in drug development and may thus be employed in pharmaceutical laboratories. These methods are both macroscopic and microscopic.

Polarized Light Microscopy

Lyotropic liquid crystals except for cubic mesophases show birefringence just like real crystals do. Birefringence can be observed in a polarization microscope. Two polarizers in cross position are mounted below and above the birefringent object being examined. The cross position of the polarizers provides plane polarized waves perpendicular to each other. Therefore, the light passing the polarizer below an isotropic object cannot pass the polarizer in across position above the object. In an anisotropic material, some parts of the light are able to pass the second polarizer because the plane polarized beam has been rotated by an angle relative to the plane of the incoming beam.

Each liquid crystal shows typical black and white textures. The addition of an λ-plate with strong birefringent properties enables the observation of color effects of the textures in yellow, turquoise, and pink. Color effects arise because rotation of the plane polarized light depends on wavelength. The thickness of the λ-plate is suited for a wavelength of 550 nm. After leaving the plate, this wavelength swings in the same plane as the incoming polarized white light does. Therefore, it is totally absorbed by the second polarizer in the cross position. All the other wavelengths of the white light except for the 550-nm one are more or less rotated with respect to the polarization plane. Hence they pass the polarizer with various intensities. White light minus the 550-nm wavelength (green yellow) gives the impression of a pink color. With an additional birefrigent liquid crystalline material in the microscope, small deviations of the wavelengths being absorbed occur; thereby, turquoise and yellow textures can be observed.

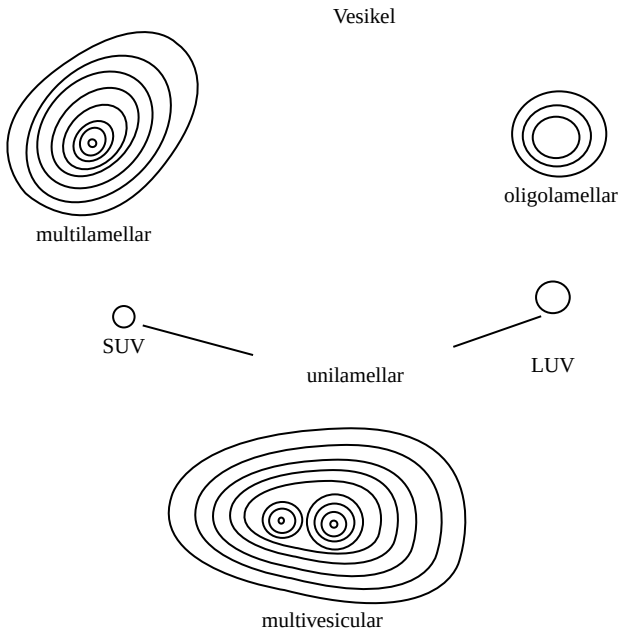


Fig. 5 Schematic cross sections of vesicles, each line represents a bilayer of hydrated molecules. (From Ref. 2.)

Hexagonal mesophases can be recognized by their typical fan-shaped texture (Fig. 7a). Lamellar mesophases typically show oily streaks with inserted maltese crosses (Fig. 7b). The latter result from defects, so-called confocal domains, that arise from concentric rearrangement of plane layers. These defects prevail in some lamellar mesophases. Hence, no oily streaks occur but maltese crosses are the dominant texture (Fig. 7c).

The smectic mesophases of the thermotropic liquid crystals show a variety of textures but resemble the fan-shaped texture of the lyotropic hexagonal mesophase. More comprehensive literature is recommended for further reading (5).

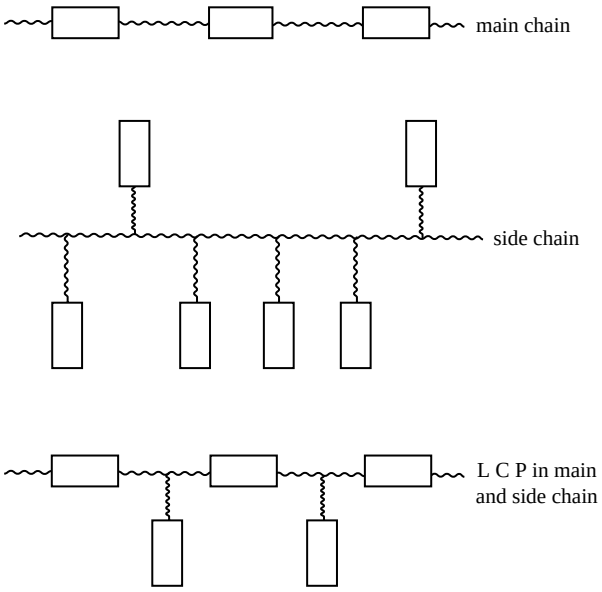


Fig. 6 Liquid crystal polymers with the mesogen within the main chain, the side chain and both the main and side chain, respectively. (From Ref. 2.)

Transmission Electron Microscopy (TEM)

Due to the high magnification power of the electron microscope, the microstructure of liquid crystals can be visualized. However, aqueous samples do not survive the high vacuum of an electron microscope without loss of water and thus their microstructure changes. Therefore, special techniques of sample preparation are necessary prior to electron microscopy. The freeze fracture technique has proven to be successful in this regard (Fig. 8). For this purpose, a replicum of the sample is produced and viewed in the electron microscope. To preserve the original microstructure of the sample during the replication, the first step is shock freeze the sample. For high freezing rates to (10^5 – 10^6 K/s), the sample is sandwiched as a thin layer between two gold plates and then shock frozen with either nitrogen-cooled liquid propane at −196°C or slush nitrogen at −210°C. If the temperature of the cooling medium is far below its boiling temperature, an efficient freezing rate can be obtained.

The frozen sample within the sample holder is transduced into the recipient of a freeze fracture apparatus, in which the fracture is performed at a temperature of −100°C and a vacuum between 10^{-6} and 5×10^{-7} bar. Within a homogeneous material, the fracture occurs by randomly because all structural elements have equal probabilities for fracturization. However, even a homogeneous material often consists of more or less polar areas. Within polar areas, stronger interactions via hydrogen bonds prevent the fracture; thus, fracture within polar areas is less probable than is fracture within apolar areas. Therefore, the sample profile obtained after

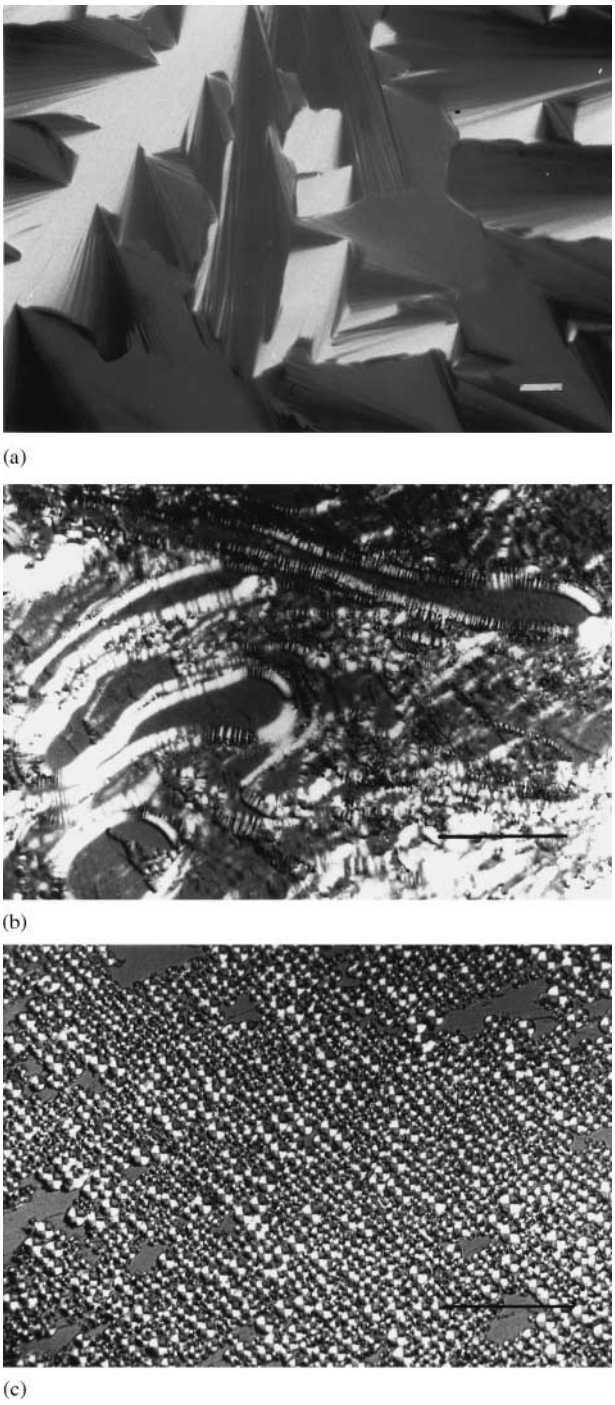


Fig. 7 Polarized light micrographs of (a) hexagonal; (b) and (c) lamellar liquid crystals. Bar 50 μm. (From Ref. 17.)

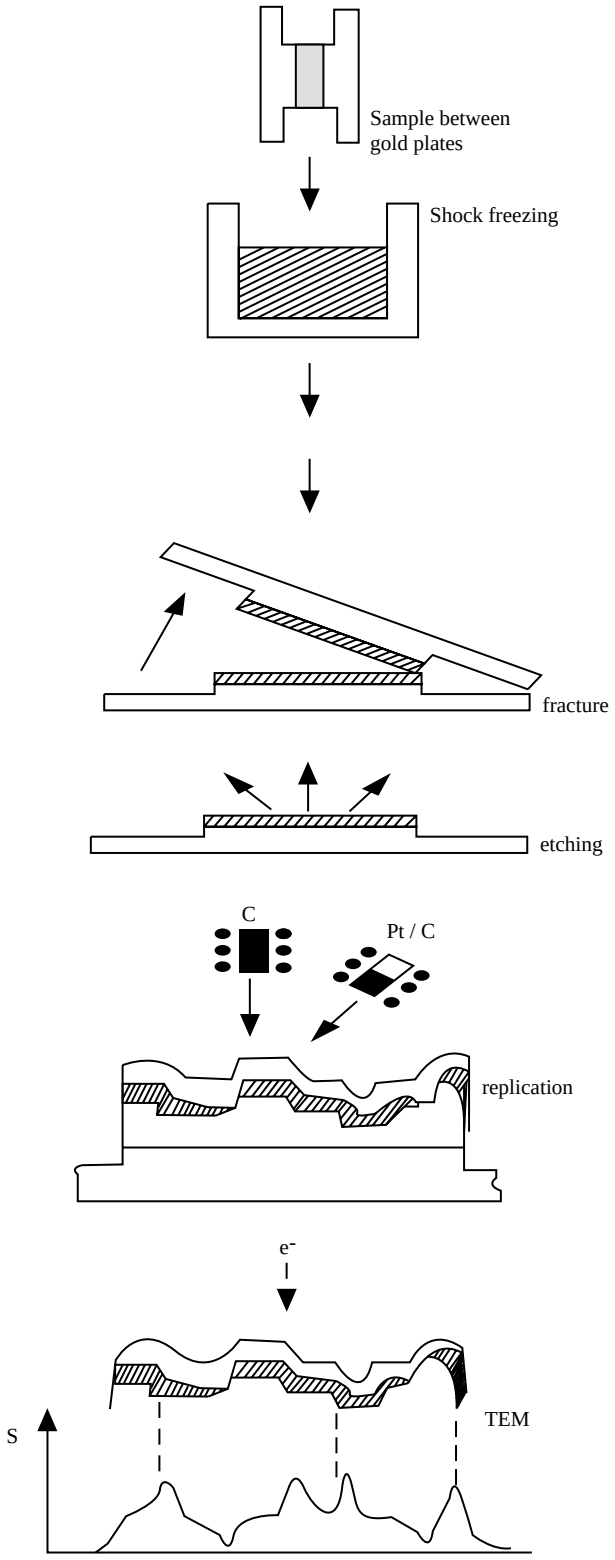


Fig. 8 Freeze fracture replication technique for transmission electron microscopy (TEM). (From Ref. 18.)

fracturization represents the microstructure of the sample just qualitatively and not quantitatively.

Immediate etching after freeze fracture provides sublimation of nonpermanent constituents (commonly ice), with the effect that level differences in the sample surface appear more pronounced.

Following this, the sample surface is shadowed with 2-nm thick platinum under an angle of 45°. Additional vertical shadowing with a 10-times thicker carbon layer of 20-nm platinum provides a high mechanical stability of the replicum, which means easier handling as regards removing, cleaning, drying, and finally observing in the transmission electron microscope (TEM).

The shadowing with platinum under an angle of 45° provides differences in contrast because platinum precipitation takes place preferably at sample positions that face the platinum source in luff whereas sample positions in lee are less or not shadowed. In the TEM, these different thicknesses of platinum absorb the electron beam to different extents, thus forming shadows. This phenomenon results in the formation of a plastic impression of the transmission electron micrographs of the replicum.

Fig. 9a–c represents transmission electron micrographs of different lyotropic liquid crystals after freeze fracture without etching. The layer structure of the lamellar mesophase, including confocal domains, hexagonal arrangement of the rod-like micelles within the hexagonal mesophase, and close by packed spherical micelles within the cubic liquid crystal can be clearly seen.

Fig. 9d and e shows aqueous dispersions of vesicles. The smaller the vesicle, the less probable is an upcoming cross fracture. Thus the question of whether the vesicle is uni- or multilamellar can probably not be answered. At least for the fluid vesicle dispersions, it is possible to solve the problem using cryo-TEM.

For this purpose, it is necessary to give sufficient contrast to a thin film of the frozen sample by using, for example, osmium tetroxide. Then the sample can directly be viewed in the TEM (at a temperature of −196°C). The adjustment of the temperature to −196°C provokes a very low vapor pressure, especially of water, so that the examination of the probe is possible by preservation of the microstructure despite the high vacuum. A disadvantage of cryo-TEM is the classification of vesicles according to their size. Due to the fluid property of vesicle dispersion prior to freezing, the thickness of the sample film varies from the center to the outside. Hence, smaller vesicles stay in the center, where the film is thin, whereas the larger ones linger at the outside margin in the thicker part of the film. In this outer part the vesicles escape detection. Hence the resulting distribution does not represent the actual size distribution.

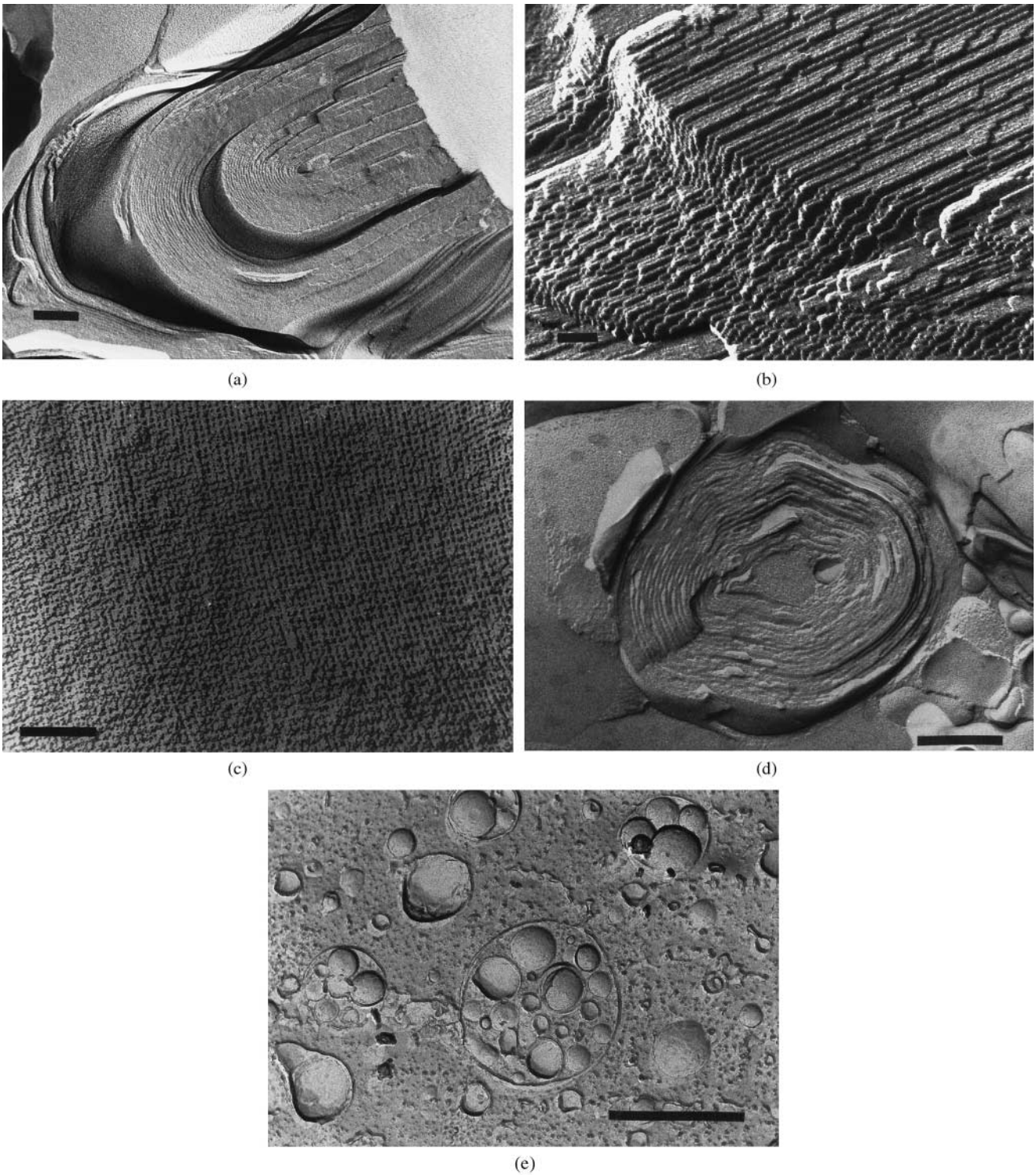


Fig. 9 Transmission electron micrographs of freeze fractured liquid crystals. (a) lamellar with confocal defects, bar 100 nm; (b) hexagonal, bar 100 nm; (c) cubic of type I, bar 100 nm; (d) multilamellar vesicle consisting of dodecyl-PEG-23-ether, cholesterol and water, bar 200 nm; (e) multivesicular vesicle, bar 1 μm. (a and b: Adapted from Ref. 19; c: Adapted from Ref. 20; d: Adapted from Refs. 21 and 22.)

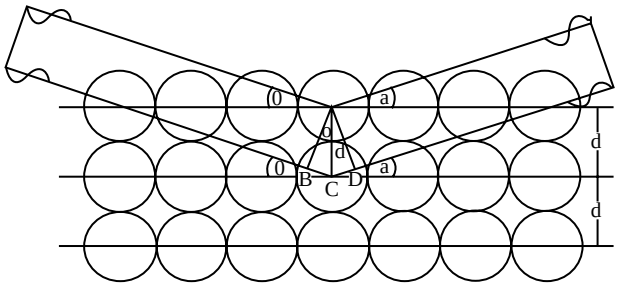


Fig. 10 Schematic representation of the reflection conditions according to Bragg's equation.

X-ray Scattering

With X-ray scattering experiments, characteristic interferences are generated from an ordered microstructure (23). A typical interference pattern arises due to specific repeat distances of the associated interlayer spacing d. By Bragg's equation, d can be calculated as $d = n(\lambda/2)\sin\vartheta$ where λ is the wavelength of the X-ray (e.g., 0.145 nm by using a copper anode or 0.229 nm by using a chromium anode), n is an integer and denotes the order of the interference and, ϑ is the angle under which interference occurs (i.e., reflection conditions are fulfilled). (See Fig. 10 for a further illustration).

Bragg's equation points at the inverse proportionality between d and ϑ. Large terms for d in the region of long-range order are registered by the small-angle X-ray diffraction technique (SAXD), whereas small terms for d in the region of short-range order are registered by the wide-angle X-ray diffraction technique (WAXD). SAXD is important for the exact determination of the distances of d of liquid crystalline systems. With WAXD, the loss of short-range order of liquid crystalline systems can be recognized in terms of the absence of interferences, which are characteristic of the crystalline state.

Interferences can be detected in two ways: (i) the film detection; and (ii) the registration of X-ray counts with scintillation counters or position-sensitive detectors.

However, SAXD does not only detect interferences from which the interlayer spacings can be calculated, but also enables to decide from the sequence of the interferences the type of liquid crystal (24, 25).

The sequence of the interferences for different liquid crystals is as follows:

Lamellar: $1 : 1/2 : 1/3 : 1/4 \ldots$
Hexagonal: $1 : 1/\sqrt{3} : 1/\sqrt{4} : 1/\sqrt{7} \ldots$
Cubic: $1 : 1/\sqrt{2} : 1/\sqrt{3} : 1/\sqrt{4} \ldots$
Cubic: $1 : 1/\sqrt{4} : 1/\sqrt{5} : 1/\sqrt{6} \ldots$

Differential Scanning Calorimetry (DSC)

Phase transitions go along with changes in energy content of the respective system. This phenomenon is caused by changing either the enthalpy ΔH or the entropy ΔS. Enthalpy changes cause endothermic or exothermic signals depending on whether the transition is due to consumption of energy (e.g., melting of a solid) or release of energy (e.g., recrystallization of an isotropic melt).

It should be mentioned that the transition from crystalline to amorphous requires much energy, whereas the transition from crystalline to liquid crystalline, from liquid crystalline to amorphous, and particularly the transition between different liquid crystals consume low amounts of energy. Therefore, care has to be taken about the appropriate sensitivity of the measuring device as well as on a sufficiently low detection limit (26).

Entropically caused phase transitions may be recognized by a change in baseline slope according to a change in the specific heat capacity. In particular, the phase transitions of liquid crystalline polymers result from entropic reasons, thus being considered transitions of the second order. These are usually called glass transitions. They can be overlaid from an enthalpic effect so that their detection might be complicated.

Rheology

Different types of liquid crystals exhibit different rheological properties (27, 28). With an increase in the microstructural organization of the liquid crystal, its consistency increases and the flow behavior becomes more viscous. The coefficient of dynamic viscosity η, although a criterion for the viscosity of just ideal viscous flow behavior (Newtonian systems), is rather high for cubic and hexagonal liquid crystals but fairly low for lamellar ones; however, the flow characteristics are not Newtonian but plastic for cubic and hexagonal crystals or pseudoplastic for lamellar ones.

For thermotropic liquid crystals, the viscosity increases in the following sequence:

nematic $<$ smectic A $<$ smectic C.

The low flowability of lyotropic liquid crystals such as cubic and hexagonal mesophases is due to their three-dimensional and two-dimensional order, respectively. Lamellar mesophases with one-dimensional long-range order have a fairly high flowability. Due to their gel character, cubic and hexagonal mesophases even exhibit a yield stress until flow occurs. Unlike the corresponding

inverse liquid crystals, the gel character is much more pronounced because of the interactions between polar functional groups located at the surface of the associates. Via polar interactions, for example hydrogen bonds, the associates may form strong networks with each other. On the other hand, the surface of the associates of inverse mesophases consists of apolar groups of the associated molecules. Thus the resulting interactions are less strong and the gel can get deformed more easily.

A mechanical oscillation measurement is the method of choice for determining the elasticity of liquid crystalline gels. Without applying a superposition of shear strain, the viscoelastic properties of liquid crystals may be studied without a change in network microstructure, which usually occurs in terms of mechanical deformation with rheological investigations. With the oscillation experiments, the viscoelastic character of cubic and hexagonal mesophases as well as that of lamellar mesophases and highly concentrated dispersions of vesicles (which also show viscoelastic behavior) can be quantified. A vesicle dispersion of low content of the inner phase, however, exhibits an ideal viscous flow property. According to the Einstein equation, η is larger than η_0 of the continuous phase, which is usually pure water or solvent, by the multi factor 2.5 × volume ratio of the dispersed phase ϕ.

$$\eta = \eta_0(1 + 2.5\varphi)$$

where is the η_0 = viscosity of pure solvent (i.e., the continuous phase) and, ϕ = volume ratio of the inner phase.

Determination of Vesicle Size by Laser Light Scattering

Vesicle size is an important parameter in not only in-process control but particularly quality assurance because the physical stability of the vesicle dispersion depends on particle size and particle size distribution. An appropriate and particularly quick method is the laser light scattering (for particle size) or diffraction (for particle size distribution). Laser light diffraction can be applied for particles >1 μm and according to the diffraction theory of Fraunhofer, refers to the proportionality between intensity of diffraction and the square of particle diameter.

Rayleigh's theory holds for particles <200 nm, which considers scattering intensity to be proportional to the sixth potency of the particle diameter. Both Fraunhofer's and Rayleigh's theories are only approximations of Mie's theory, which claims that scattering intensity depends on the scattering angle, absorption, and size of the particles as well as on the refractive indices of both the particles and the dispersion medium. Unfortunately, the latter parameters are difficult to be determined. Furthermore, most vesicle dispersions consist of a dispersed mesophase with particle sizes <200 nm up to 1 μm. Therefore, photon correlation spectroscopy (PCS) that is based on laser light scattering provides an appropriate method of investigation (29).

Dynamically raised processes in the dispersion, such as Brownian molecular motion, cause variations in the intensities of the scattered light with time, which is measured by PCS. Smaller the particle, higher the fluctuations by Brownian motion. Thus, a correlation between the different intensities measured is only possible for short time intervals. In a monodisperse system following first-order kinetics, the autocorrelation function decreases rather fast. In a half logarithmic plot of the autocorrelation function, the slope of the graph enables the calculation of the hydrodynamic radius by the Stokes–Einstein equation. With the commercial PCS devices the *z*-average is determined, which corresponds to the hydrodynamic radius.

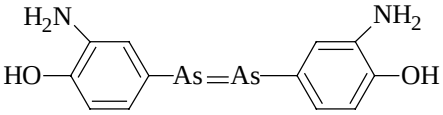
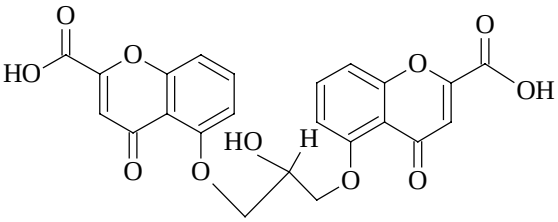
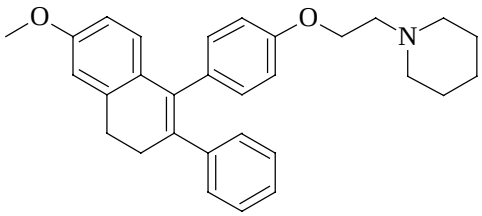
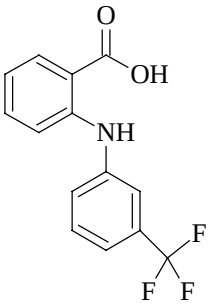
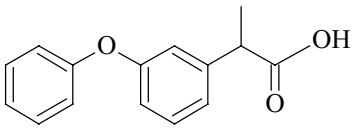
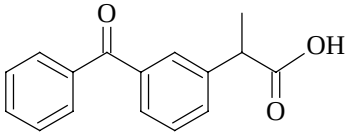
In a polydisperse system, the calculation of particle size distribution is possible, in addition, by using special transformation algorithms. For this, certain requirements need to be fulfilled: a spherical particle shape, sufficient dilution, and a large difference between the refractive indices of the inner and outer phase. As not all requirements can be usually fulfilled, the *z*-average as a directly accessible parameter is preferred to the distribution function depending on models.

APPLICATIONS OF LIQUID CRYSTALS IN DRUG DELIVERY

Liquid Crystalline Drug Substances

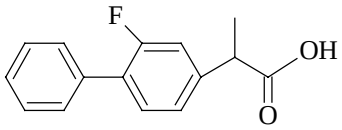
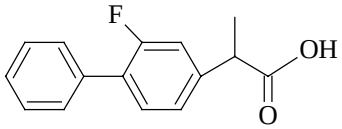
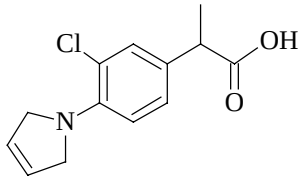
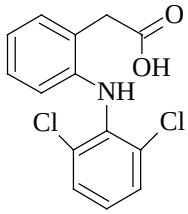
Some drug substances are able to form mesophases either with solvent or alone (30–37). In the latter case, an increase in temperature causes transition from the solid state to the liquid crystalline one. This is called thermotropic mesomorphism. Lyotropic mesomorphism occurs in combination with a solvent, usually water. Furthermore, a change in temperature may cause additional transitions. Thermotropic and/or lyotropic liquid crystalline mesophases of drug substances may interact with mesomorphous vehicles as well as with liquid crystalline structures in humans. Table 2 presents drug substances for which either thermotropic or lyotropic mesomorphism has been proven.

Table 2 Liquid crystalline drug substances

Drug	Type of liquid crystal	Formula	Reference
Arsphenamine	Nematic		30
Disodium cromoglicinate	Nematic, hexagonal		31
Nafoxidin-HCl	Hexagonal, cubic, lamellar		32
Diethylammonium flufenamate	Lamellar		33
NSAID salts			
Fenoprofen	Lamellar		34
Ketoprofen	Lamellar		34

(Continued)

Table 2 Liquid crystalline drug substances (*Continued*)

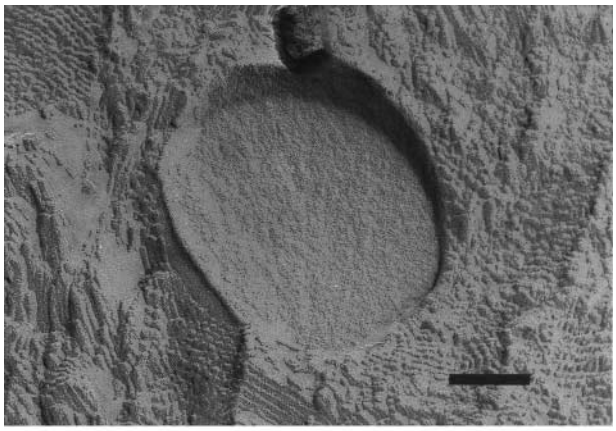
Drug	Type of liquid crystal	Formula	Reference
Ibuprofen	Lamellar		34
Flurbiprofen	Lamellar		34
Pirprofen	Lamellar		34
Diclofenac	Lamellar		36
Peptide hormone	LH-RH analogue		35

Arsphenamin was the first drug substance with thermotropic mesomorphism (30) to be therapeutically used as Salvarsan during the first half of the nineteenth century. The drug is effective against microorganisms and thus offered for the first time an efficient therapy of veneral diseases such as syphilis. Nowadays, it has been replaced by antibiotics with less serious side effects.

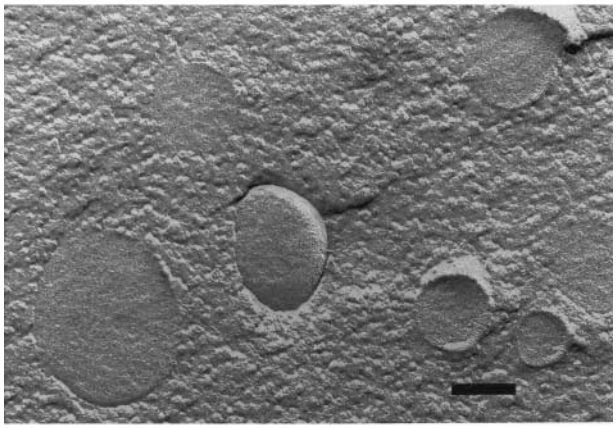
The molecular structure of arsphenamin is typical of a thermotropic mesogen. With its symmetrical arrangement of atoms, the same holds for disodium cromoglicinate (DNCG) (30), which forms thermotropic liquid crystals and additionally lyotropic mesophases with water. If micronized DNCG powder is applied to the mucosa of the nose or the bronchi, the powder will absorb water from the high relative humidity of the respiratory tract, and first transform into a lyotropic mesophase and then into a solution.

DNCG serves as a mast-cell stabilizer. Mast cells are located on the mucosa of the respiratory tract and act by releasing the mediator substance histamine on contact with an allergen, provided the patient had been sensitized previously. Due to its mast-cell stabilizing effect, DNCG acts as a prophylactic against allergic reactions associated with asthma and hay fever. In addition to this prophylactic effect, DNCG exhibits a second mode of action in causal therapy of asthma, which has not yet been fully clarified. According to recent findings, DNCG has a positive effect on the inflammation of the mucosa of bronchi.

For therapeutic purposes, a similar frequently used group of drug compounds is the nonsteroidal anti-inflammatory drugs (NSAIDs). One of the best known representatives of the aryl acetic acid derivatives in diclofenac and that of aryl propionic acid derivatives ibuprofen. As both have acidic properties, they dissociate while being dissolved and may form salts with amphiphilic properties. Together with appropriate



(a)



(b)

Fig. 11 Transmission electron micrographs of freeze fractured oily droplets dispersed in (a) a hexagonal and (b) a cubic liquid crystalline phase, bar represents 100 nm. (From Ref. 38.)

counter ions, these amphiphilic organic acids may form lyotropic mesophases with water at even room or body temperature e.g., diclofenac diethylamine or ibuprofen lysinate (34, 36). Furthermore, some anhydrates of NSAID, e.g., fenoprofen calcium (37), exhibit thermotropic mesomorphism after thermal dehydration of the crystalline salt.

All the other drugs substance listed in Table 2 have not yet been used for therapeutical purposes.

Liquid Crystalline Formulations for Dermal Application

As long as drug molecules with amphiphilic character form lyotropic mesophases, amphiphilic excipients in drug formulations form lyotropic liquid crystals. Especially surfactants, which are commonly used as emulsifiers in dermal formulations, associate to micelles after dissolution

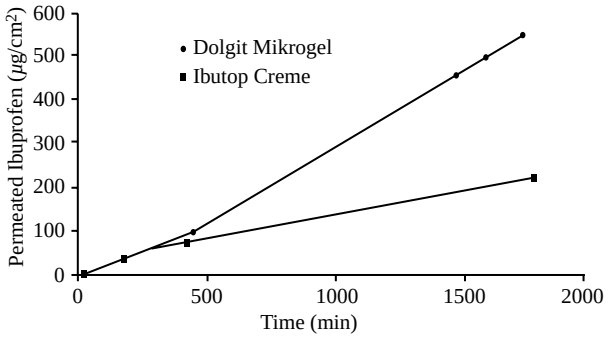


Fig. 12 Permeability of ibuprofen from different formulations via excised human stratum corneum. (Redrawn from Ref. 39.)

in a solvent. With increasing concentration of these micelles, the probability of interaction between these micelles increases, formating liquid crystals.

Surfactant gels

The use of monophasic systems of lyotropic liquid crystals is relatively seldom and is limited to gels. A variety of polar surfactants (e.g., ethoxylated fatty alcohols) are hydrated in presence of water and form spherical or ellipsoidal micelles. At high surfactant concentrations, these associates are densely packed and are thus identified as cubic liquid crystals (20).

Fig. 9c represents a transmission electron micrograph of a liquid crystalline surfactant gel of this type. Such gels are optically transparent. If agitated mechanically, their elastic properties become evident. Due to resonance effects in the audible range, they are also called ringing gels. The lipophilic components are solubilized together with the active ingredients in hydrated associates of the surfactants. However, the solubilization capacity for lipophilic components is generally limited. By exceeding this capacity, the excess of the lipophilic component will be dispersed dropwise in the liquid crystalline phase (Fig. 11b). Such systems exhibit a white appearance according to the change in refractive index at the interface between continuous liquid crystalline and dispersed oil phase. Besides, the dispersed drops are mechanically stabilized because the liquid crystalline phase of either hexagonal (Fig. 11a) or cubic character (Fig. 11c) has a high yield stress.

Ringing gels with cubic liquid crystalline microstructure are used as commercial drug formulations especially for topical NSAID formulations. Examples in the German market include Contrheuma Gel Forte N, Trauma-Dolgit Gel, and Dolgit Mikrogel. Dolgit Mikrogel was introduced in 1996 and contains ibuprofen as an active ingredient. On the one hand the high surfactant concentration of such gels

is necessary to verify the liquid crystalline microstructure; on the other hand, this concentration influences the microstructure of the stratum corneum lipids via increase in permeability. This effect is also achieved by alcohol, which is also solubilized in the formulation. Fig. 12 shows the result of permeation tests with excised human stratum corneum. The amount of ibuprofen permeating per unit time and surface area is much higher for Dolgit Mikrogel than for an aqueous mixed micellar solution of the drug. Although relatively high permeation rates are possible for the liquid preparation, the commercial formulation is significantly more effective because the high surfactant content and the alcohol favor high permeability.

A ringing surfactant gel of liquid crystalline microstructure containing the antimycotic bifonazole (Bifomyk gel) was introduced in 1995 into the German market. Similar to surfactant gels containing NSAID, an improved penetration of the active ingredient is desired in antifungal therapy of the dermis as well. However, because the liquid crystal structure only forms with a relatively high surfactant concentration, the positive effect of improved penetration must be considered together with the potential of irritation. The objective is to achieve improved penetration with minimum irritation, via a change in skin structure. Because hyphae fungal (mycelium) can penetrate deep into the epidermal layers by sliding past corneocytes of the horny layer, improvement of antimycotic therapy is of particular importance. The same holds true for penetration of NSAIDs through several epidermal layers because they have to arrive at the deeply located muscle and joint tissue.

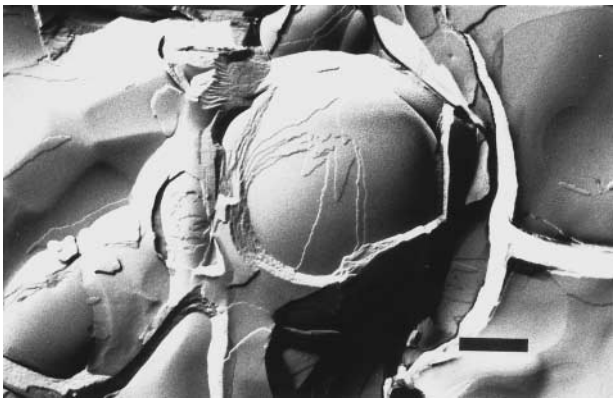


Fig. 13 Transmission electron micrograph of a freeze fractured w/o cream. The aqueous phase is dispersed as droplets within the continuous lipophilic phase and the interface consists of multiple bilayers of hydrated surfactant molecules; bar 500 nm. (From Ref. 41.)

Ointments and creams

Usually the surfactant concentration in ointments and creams is significantly lower than in surfactant gels. Ointments are nonaqueous preparations, whereas creams result from ointments by adding water. The microstructure of both ointments and creams may consist of liquid crystals, as long as a liquid crystalline network or matrix is formed by amphiphilic molecules. In a liquid crystalline matrix, it is easier to deform the system by shear; such formulations show plastic and thixotropic flow behavior on shear. In comparison to systems with a crystalline matrix which are usually destroyed irreversibly by shear, those with a liquid crystalline matrix exhibit a short regeneration time of the sheared matrix. To obtain a liquid crystalline matrix, amphiphilic surfactants, that form lyotropic liquid crystals at room temperature have to be selected. Preferably, lamellar liquid crystals that are able to solubilize high amounts of further ingredients and spread through the whole formulation as a network-forming crosslinked matrix should be formed. In contrast, ointments that contain long-chain fatty alcohols such as cetyl and/or stearyl alcohol have a crystalline structure at room temperature (40).

Although the so-called α-Phase of the fatty alcohols—a thermotropic type smectic B liquid crystal with hexagonal arrangement of molecules within the double layers—is initially formed from the melt during the manufacturing process, it normally transforms into a crystalline modification as it cools. However, the crystallization of the gel matrix can be avoided if the α-Phase can be kept stable as it cools to room temperature. This can be achieved by combining appropriate surfactants such as myristyl or lauryl alcohol and cholesterol, a mixture of which forms a lamellar liquid crystal at room temperature (41). Due to depression of the melting point, the phase transition temperature of crystalline to liquid crystalline as well as liquid crystalline to isotropic decreases. Therefore, a liquid crystalline microstructure is obtained at room temperature.

The polar character of a surfactant molecule enables the addition of water to form creams. Depending on whether the surfactant or the surfactant mixture has a strong or weak polar character, creams of type o/w or w/o are formed. Creams of w/o type are produced from systems that are stabilized solely with weakly polar surfactants such as fatty alcohols, cholesterol, glycerol monostearate, or sorbitan fatty acid esters. The surfactant or surfactant mixtures are adsorbed at the interface of the dispersed aqueous and the continuous lipophilic phase. Even multiple layers of the surfactant will be adsorbed if the concentration of mesogenic molecules is

high enough to form their own liquid crystalline phase (Fig. 13). Apart from the reduction of surface tension and/or surface energy, the liquid crystalline interface also has a mechanically stabilizing effect on the emulsion drops.

Surfactants such as sulfated fatty alcohols may be hydrated to a higher extent than the fatty alcohols alone, and thus stabilize o/w emulsions. The combination of an anionic and a nonionic surfactant has proven to be particularly effective, as the electrostatic repulsion forces among the ionic surfactant molecules at the interface are reduced by the incorporation of nonionic molecules, thereby improving emulsion stability. The combination of cetyl/stearyl sulfate (Lanette E) and cetyl/stearyl alcohol (Lanette O) to yield an emulsifying cetyl/stearyl alcohol (Lanette N) is an example of this approach. The polar properties of this surfactant mixture are dominant; therefore, o/w creams are formed. In contrast to w/o systems, the stabilizing effect of the surfactant mixture is not mainly due to adsorption at the interface. Instead, the mixed surfactants are highly hydrated and form a lamellar network, which is dispersed throughout the continuous aqueous phase, whereas the dispersed lipophilic components are immobilized within the gel network. However, this hydrated gel matrix is not crystalline at room temperature as are the corresponding w/o creams with cetyl/stearyl alcohol, but is in its α-phase, which belongs to the thermotropic smectic liquid crystals and exhibits a strong similarity to lyotropic lamellar liquid crystals.

Analogous gel matrices of liquid crystalline lamellar phases can also be formed with nonionic mesogens, for example, with the combination of cetyl/stearyl alcohol and ethoxylated fatty alcohol, provided the hydrophilic and lipophilic properties of the surfactant molecules are more or less balanced to favor the formation of lamellar structures.

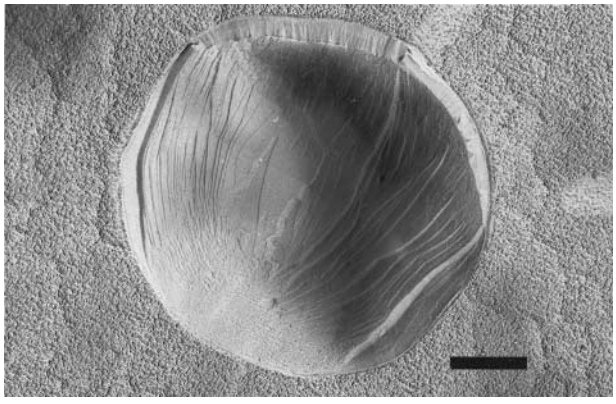


Fig. 14 Transmission electron micrograph of Voltaren Emulgel. The interface between the continuous hydrogel and the dispersed emulsion droplets consists of multiple bilayers of hydrated surfactant molecules bar 500 nm. (From Ref. 44.)

Liposome Dispersions

Although liposomes have been extensively studied since 1970, only a few commercial drug formulations contain liposomes as drug carriers (42, 43). The first commercial drug formulation with liposomes for topical administration was registered in Italy. The antimycotic econazol was encapsulated in liposomes being dispersed in a hydrogel (Ecosom Liposomengel, formerly Pevaryl Lipogel). Due to the formation of a highly hydrated gel network of the hydrophilic polymers, liposomes are immobilized within the gel network and thus mechanically stabilized. This stabilization via gelation of the continuous aqueous phase can also be applied to other dispersion systems (e.g., suspensions or emulsions). An example of such an emulsion/hydrogel combination that contains heparin sodium as an active ingredient and liposomes as the additional dispersed phase (the latter only since 1995) is Hepaplus Liposom. A formulation with an analogous emulsion/hydrogel combination but without additional liposomes is Voltaren Emulgel. The transmission electron micrograph (Fig. 14) reveals an adsorption of lamellar liquid crystals at the interface of dispersed oil drops and the aqueous continuous phase. The aqueous continuous phase is again a hydrogel based on polyacrylate in which the lipophilic phase is immobilized. The interface consists of multilamellar layers consisting of both surfactant and drug molecules. Thus the hydrogel is not only stabilized by the hydrogel network itself but also by the liquid crystalline interface, which provides an additional stabilization. The active ingredient diclofenac diethylamine diffuses slowly from the dispersed phase via the multilamellar interface into the continuous phase, from where it penetrates into the epidermis.

Similar to Voltaren Emulgel, oily droplets of an eutectic mixture of lidocaine and prilocaine are dispersed in a hydrogel to provide local anaesthesia of the skin for injections and surgical treatment (Emla cream). A further possibility is the dermal administration of a liposome dispersion as a spray (Heparin PUR ratiopharm Sprühgel). After administration, water and isopropyl alcohol evaporate partially to result in an increase of concentration and thereby in a transition from the initial liposome dispersion to a lamellar liquid crystal (45). The therapeutic effect thus appears to be influenced favorably by the presence of lecithins alone, rather than by the degree of dispersion of liposomes.

Liposome dispersions for parenteral administration

Depending on their size and surface charge, parenterally administered liposomes interact with the reticulo endothelial system (RES) and provoke an immunological response. After being marked by the adsorption of certain serum proteins, called opsonins, they are identified as an invader and destroyed by specific immune cells mainly in the liver, spleen, and bone marrow.

This passive drug targeting enables an efficient therapy of diseases of these organs or their affected cells. Clinical tests in the therapy of parasitic diseases of the liver and spleen have proven most efficient by having encapsulated the drug substance in liposomes. Apart from passive drug targeting, drug encapsulation within liposomes offers a modification of the therapeutical effect in terms of intensity and duration, together with a minimization of undesired side effects. For this purpose, liposomes have to circulate for as long as possible in the vascular system and remain unrecognized by phagocytic cells.

The antimycotic amphotericine is encapsulated in liposomes and marketed as AmBisome to treat severe systemic mycosis. The liposomal encapsulation reduces the toxicity of amphotericine while increasing half-life of the drug and plasma level peaks (43). Due to stability reasons, the parenteral formulation is a lyophilized powder, which has to be reconstituted by adding the solvent just before administration.

The cytostatic daunorubine, which is administered in the later state of Kaposi sarcoma of AIDS patients, is encapsulated in liposomes of about 45 nm size (43). The liposome dispersion is marketed as a sterile, pyrogen-free concentrate (DaunoXome) and has to be diluted with a 5% glucose solution just before being administered as an infusion. Although daunorubicine by itself is cardiotoxic, the liposomal formulation attacks cardiac tissue only insignificantly, but strongly affects the tumor cells by being taken up preferably. It is postulated that small unilamellar vesicles (SUV) may pass through endothelial gaps in recently formed capillaries of the tumor, thereby entering the tumor tissue. At this site, the drug is released from the liposomal carrier and inhibits the proliferation of the tumor cells.

Liposome dispersions for an installation into the lung

A liposomal formulation consisting of a surfactant, which usually coats the mucosa of the bronchi and prevents a collapse of the alveolar vesicles of the lung, has been developed for patients who suffer either from infant respiratory distress syndrome (IRDS) or adult/acquired respiratory distress syndrome (ARDS). IRDS often affects prematurely born babies who have not yet developed a functional lung surfactant and therefore develop a failure in pulmonary gas exchange. ARDS is also a life-threatening failure/loss of the lung function, usually acquired by illness or accident. Clinical trials with liposomal surfactant have proven to be efficient in prophylactic treatment of IRDS and ARDS.

The surfactant is obtained by extraction from the lungs of cattle, by washing and centrifuging several times. This raw extract is treated with appropriate organic solvents, sterilized by filtration, dried by solvent evaporation under aseptic conditions, resuspended in water, and finally homogenized in a French press under cooling. Care has to be taken to maintain sterility of the extract during all procedures. Special attention has to be paid to transmissible spongiform encephalopathies (TSE). The whole manufacturing process has been validated in terms of a decrease of infectious material by a factor of 10^{21}, although a factor of 10^{8} is sufficient. The result is a formulation (Alvefact) that is considered safe in context of TSE and viruses, and it also contains all relevant components of a lung surfactant in terms of pulmonary exchange of gas (43).

Transdermal Patches

To obtain a systemic effect via percutaneous penetration of a drug compound, a high permeability through the stratum corneum and the living tissue beneath is required as well as a high potency of the drug for a low dose to be administered. For an additional short biological half-life, the development of controlled release transdermal systems is a good choice.

Transdermal patches are high-tech devices, which contain the drug substance in a reservoir from which the drug is released in a controlled manner (i.e. zero-order kinetics). The control element is either a membrane or a matrix. Membrane-controlled patches were the first to be marketed. A major disadvantage of these is the so-called dose dumping that occurs in case of a membrane damage during handling. To ensure the desired drug control, even liquid crystalline polymers have been examined with regard to their usefulness in membrane-controlled transdermal patches (46). The matrix-controlled transdermal patch consists of only one functional element, the porous polymer matrix, which not only controls drug release but simultaneously acts as a drug reservoir and adhesive element.

Transdermal patches are marketed worldwide with the drug substances glycerole trinitrate, estradiol,

testosterone, clonidine, scopolamine, fentanyl and nicotine, respectively. The patch has to remain for up to one week at the appropiate body site. In this case the drug amount in the reservoir is rather high. As liquid crystalline vehicles with lamellar microstructure have high solubilization capacities, they are recommended as reservoirs for transdermal patches (47), although the high surfactant concentration of the lamellar liquid crystal might have a irritate the skin. Especially in terms of the membrane-controlled patch, the liquid crystalline vehicle is not in direct contact with the skin and thus will not exhibit an irritating effect on the skin.

Sustained Drug Release from Solid, Semisolid, and Liquid Formulations

The therapy of a chronic desease requires a repeated dosing of a drug. Drugs having a short biological half-life have to be administered up to several times daily within short intervals. To reduce the application frequency, sustained formulations have been developed. Liquid crystalline excipients are appropiate candidates for this because in a liquid crystalline vehicle the drug diffusion is reduced by a factor of 10 to 1000 in comparison with a liquid vehicle such as a solution (48–50). The factor depends on the kind of liquid crystal being employed.

Solid formulations

Solid formulations for sustained drug release may contain mesogenic polymers as excipients. The mesogenic polymers form a matrix, which is usually compressed into tablets. Some of the most frequently used excipients for sustained release matrices include cellulose derivatives, which behave like lyotropic liquid crystals when they are gradually dissolved in aqueous media. Cellulose derivatives such as hydroxy-propyl cellulose or hydroxy-propylmethyl cellulose form gel-like lyotropic mesophases in contact with water (51), through which diffusion takes place relatively slowly. Increasing dilution of the mesophase with water transforms the mesophase to a highly viscous slime and then to a colloidal polymer solution.

Semisolid formulations

The solubilization of a drug substance in monophasic liquid crystalline vehicles results in semisolid formulations, which are preferably used for topical application. (See the sections on Surfactant Gels and Transdermal Patches.)

Liquid formulations

Sustained release from disperse systems such as emulsions and suspensions can be achieved by the adsorption of appropriate mesogenic molecules at the interface. The drug substance which either forms the inner phase or is included in the dispersed phase cannot pass the liquid crystals at the interface easily and thus diffuses slowly into the continuous phase and from there further into the organism via the site of application. Such as sustained drug release is especially pronounced in the case of multilamellar liquid crystals at the interface.

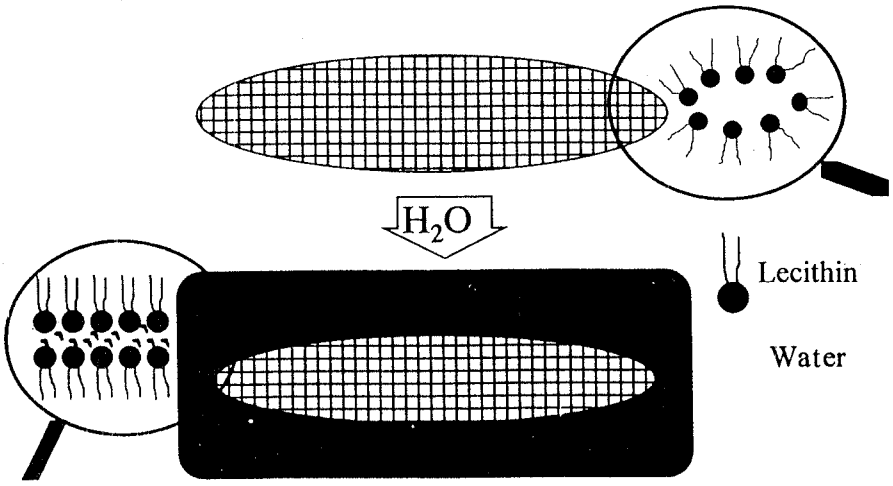


Fig. 15 Application induced transformation of a reverse micellar solution into a liquid crystal on contact with aqueous media. (From Ref. 10.)

A further possibility is the formation of liquid crystals on contact with body fluids at the site of application. The applied drug solution interacts with body fluids such as plasma, tear, fluid, or skin lipids and undergoes a phase transition to a mono- or multiphasic system of liquid crystals (Fig. 15). For example oily solutions of reverse micellar solutions of phospholipids, which solubilize any additional drug, transform into liquid crystalline lamellar phases by the absorption of water when applied to the mucosa. Drug release is controlled by the liquid crystals because diffusion within the liquid crystalline phase is slowest and thus rate-controlling (50). This principle can be used for ophthalmological administration as well as for nasal, buccal, rectal, vaginal, or even parenteral subcutaneous application (52). However, the peroral administration of such reverse micellar solutions either directly or encapsulated within soft gelatin capsules is not recommended (53) because the sustained release effect is limited by interindividual variations in digestion, such as the amount and composition of the gastric fluid as well as its ability to emulsify and solubilize in terms of enteral absorption.

For the treatment of paradontitis of infected gum pockets, the chemotherapeutic metronidazol has proven to be effective. The crystalline prodrug metronidazol-benzoate, which has to provide the active metronidazol through dissolution and hydrolysis, is suspended in an oleogel (Elyzol Dentalgel). The oleogel consists of glycerol monooleate and sesame oil, which are immobilized within the matrix structure of the surfactant. The base melts at body temperature and spreads evenly over the inner surface of the gum pockets. The molten system absorbs water and transforms into a reverse hexagonal phase. This liquid crystalline structure has a high viscosity. The resulting system adheres well to the surface of the mucosa and releases the active ingredient slowly (10).

Liquid Crystals in Cosmetics

Liquid crystals are mainly used for decorative purposes in cosmetics. Cholesteric liquid crystals are particularly suitable due to their iridescent color effects, and find applications as coloring for nail varnishes, eye shadows, and lipsticks. The structure of these thermotropic liquid crystals changes with body temperature, resulting in the required color effect. In recent times, such thermotropic cholesteric liquid crystals have also been included in body care cosmetics, where they are dispersed in a hydrogel. Depending on whether this dispersion in the hydrogel involves stirring or a special spraying process, the iridescent liquid crystalline particles are distributed statistically in the gel (Estée Lauder Time Zone Moisture Recharging Complex) or concentrated locally (Vichy Restructure Contour des Yeux) to give the formulation the required appearance. Tests of the cosmetic efficiency of the liquid crystalline constituents have not yet been published.

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